

Mass Spectrometric Immunoassay for Parathyroid Hormone Related Protein (PTHrP)

*K. Zheng, J.D. Rivera, J.S. Vogel, B.A. Buchholz, D.W.
Burton, L.J. Deftos, D.A. Herold and R.L. Fitzgerald*

*This article was submitted to
48th ASMS Conference on Mass Spectrometry and Allied Topics
Long Beach, CA
June 11-15, 2000*

U.S. Department of Energy

Lawrence
Livermore
National
Laboratory

June 16, 2000

DISCLAIMER

This document was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor the University of California nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or the University of California, and shall not be used for advertising or product endorsement purposes.

This is a preprint of a paper intended for publication in a journal or proceedings. Since changes may be made before publication, this preprint is made available with the understanding that it will not be cited or reproduced without the permission of the author.

This report has been reproduced
directly from the best available copy.

Available to DOE and DOE contractors from the
Office of Scientific and Technical Information
P.O. Box 62, Oak Ridge, TN 37831
Prices available from (423) 576-8401
<http://apollo.osti.gov/bridge/>

Available to the public from the
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Rd.,
Springfield, VA 22161
<http://www.ntis.gov/>

OR

Lawrence Livermore National Laboratory
Technical Information Department's Digital Library
<http://www.llnl.gov/tid/Library.html>

Mass spectrometric immunoassay for parathyroid hormone related protein (PTHrP)

Kefei Zheng¹, Jeffery D. Rivera¹, John S. Vogel², Bruce A. Buchholz², Douglas W. Burton¹, Leonard J. Deftos¹, David A. Herold¹ and Robert L. Fitzgerald¹

¹VA Healthcare System, San Diego and University of California-San Diego, San Diego, CA.

²Lawrence Livermore National Laboratory, Livermore, CA.

Introduction

Many cancers, including prostate, breast and lung express parathyroid hormone related protein (PTHrP). Despite the common tumor overexpression of PTHrP, serum levels of PTHrP are not commonly elevated in affected patients. We postulate that the reasons for the discrepancy between tissue and serum measurements of PTHrP are the inadequate sensitivity and specificity of current PTHrP serum assays. To improve the clinical value of PTHrP serum assays for the cancer patient, we are developing a new generation of novel and ultrasensitive PTHrP serum immunoassays based on immunoaffinity purification, nanospray liquid chromatography tandem mass spectrometry (LC/MS/MS) and accelerator mass spectrometry (AMS).

Methods and Instrumentation

LC/MS/MS (Finnigan LCQ) equipped with a nanospray interface we built is being used to identify cancer-specific forms of PTHrP. Based on the LC/MSMS data, a two site immunoassay to quantify PTHrP and related peptides is being developed. The immunoassay uses a monoclonal capture antibody and a biotinylated secondary antibody. Streptavidin labeled with ¹⁴C is the detection signal. After removing non-specifically bound ¹⁴C, the radiolabel is then analyzed using AMS (Lawrence Livermore National Laboratory) to determine the amount of PTHrP initially present.

Preliminary Data

Using the nanospray interface, the signal to noise was greater than 100:1 for 120 fmoles of PTHrP67-86, demonstrating our ability to identify the femtomolar amounts of these peptides expected in biological specimens. PTHrP140-173 and a mutant form of PTHrP termed D5 were incubated with a cellular extract from an immortalized human cell line. The native proteases in the cellular extract processed nearly all of the parent PTHrP peptides. Analyses using LC/MS/MS showed distinct peptide maps for PTHrP140-173 and the D5 mutant. The y and b ion series correctly identified the initial sequence of the mutant as TALLWGLGQK vs. the native form of TALLWGLK. In order to sequence endogenous peptides present in biological samples, we have developed a series of antibodies covering the entire linear portion of PTHrP and linked them to Sepharose beads so they can be used to immunoaffinity purify the peptides of interest. The two antibodies with the highest affinity (R115 and R1793) were used to isolate PTHrP from serum samples that had been supplemented with PTHrP1-34 and PTHrP107-139. R115 recognizes the amino-terminal peptide (1-34) and R1793 recognizes the carboxy-terminal peptide (109-141). LC/MS/MS analysis of the immunoaffinity purified peptides showed that R115 and R1793 isolated peptides with spectra consistent with PTHrP1-34 and PTHrP107-139

respectively. This demonstrates our ability to isolate specific peptides from a complex biological matrix that could subsequently be identified using LC/MS/MS. We exploited the attomole

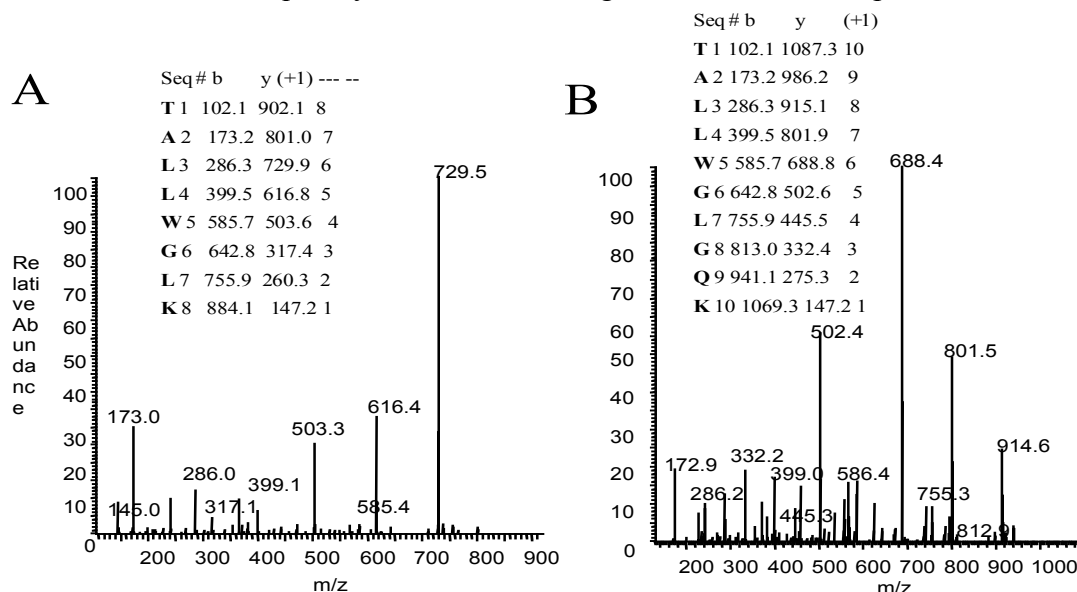


Figure 1. Product ion spectra of A) Initial amino acids of PTHrP140-173. B) initial amino acids of D5 synthetic variant.

sensitivity of ^{14}C -AMS by labeling streptavidin with ^{14}C formaldehyde. A standard curve for PTHrP 1-141 was generated from 0 to 1.3 pmol/mL using AMS and the two site immunoassay routinely performed in our laboratory. These results showed that mass spectrometry can be used in combination with immunological procedures to isolate, identify, and quantify PTHrP and other biologically related peptides.

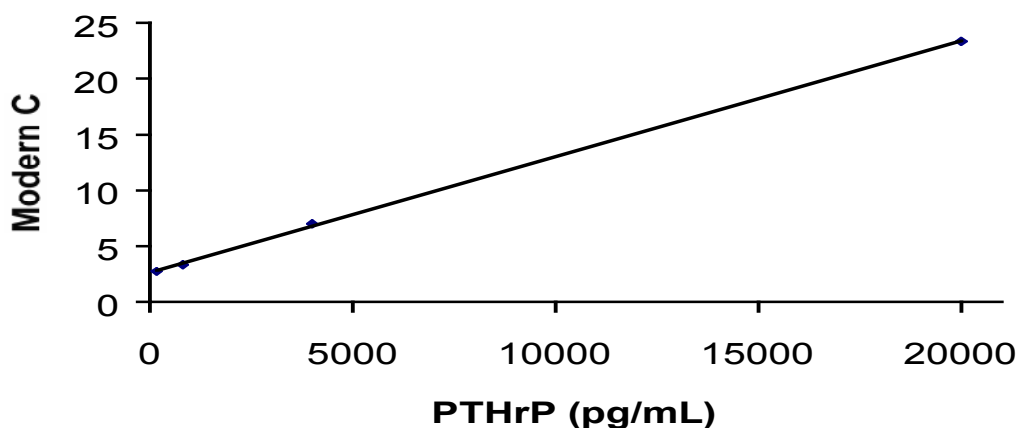


Figure 2. Mass spectrometric immunoassay for PTHrP. Standard curve for PTHrP 1-141 determined with two site immunoassay using ^{14}C labeled streptavidin and Accelerator Mass Spectrometry (N=3, each data point). The limit of detection of this initial experiment is 160 pg/mL.

Novel Aspect

MS can be used to develop sensitive and specific PTHrP immunoassays to improve the clinical management of patients with PTHrP producing cancers.

This work was performed in part under the auspices of the U.S. Department of Energy by University of California Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48.